CONCENTRATION OF ANTISTREPTOCOCCIC AND ANTIGONOCOCCIC SERA.*

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Antistreptococcic sera and antigonococcic sera have been on the market for some time, but doubtful success, and in some instances, failure, have attended their use. The poor results may be due to several causes, among which low potency is possibly an important factor. We therefore concentrated these sera according to the methods used for the concentration of diphtheria antitoxin. It has been shown[1] that a serum for Rocky Mountain spotted fever can be concentrated by precipitating the pseudoglobulin fraction and that the antibodies are united chiefly to this fraction. The antistreptococcus sera for this purpose were obtained from horses immunized with 36 strains of streptococci under the direction of Dr. D. J. Davis and Professor H. G. Wells, and the antigonococcus serum was obtained through the courtesy of Parke, Davis & Co., in Detroit, Mich.

Diphtheria antitoxin is concentrated usually by the methods devised by Gibson and Banzhaf. The modification introduced by Banzhaf consists in heating the plasma, obtained by drawing blood into a solution of sodium citrate or potassium oxalate, for a period of about 15 hours at 56° C. The plasma is then diluted with about one-half its volume of water and enough saturated ammonium sulphate solution added to make a 30 per cent concentration. The precipitate is filtered off, redissolved in water, and the solution saturated with sodium chloride. This saturated solution is filtered and the globulins precipitated from the filtrate with 2.75 c.c. acteic acid per liter. The precipitate is then gathered on paper filters, pressed out to remove mechanically as much of the liquid as possible, and the precipitate, now forming a solid cake, dialyzed against running water. After the precipitate has gone into solution,

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enough sodium carbonate is added to make the reaction amphoteric. In about seven to nine days the ammonium sulphate and sodium chloride are dialyzed out and the globulin solution is filtered, first through pulp of filter paper and then through a Berkefeld filter for sterilization.

The filtrate remaining from the first precipitate is mixed with enough ammonium sulphate solution to make the concentration 55 per cent. The resulting precipitate is gathered on paper filters, pressed out, and dialyzed in the same manner as the first precipitate. In this case, however, it is not necessary to neutralize the globulin solution.

The loss of potency by heating antidiphtheritic serum previous to precipitation is slight. The advantages of heating are twofold. There seems to be a rearrangement of the relation of the constituents of the serum on heating so that a part of the superfluous protein is rendered insoluble and consequently a higher concentration of antibodies is obtained. Heat also destroys toxic substances which cause local reactions after injection. Serum rashes are less common if the serum has been heated previous to precipitation. We considered that it might be well, however, to try different methods of concentration, to be able to judge the effects by the results.

The tests for potency of antistreptococcic and antigonococcic sera were made by using the opsonic index, in default of more exact methods. The following routine was carried out in determining the potency of the original sera and the concentrated sera.

The leukocytes were obtained from blood drawn from the ear of the operator into a sterile centrifuge tube containing 2 per cent sodium citrate solution. The blood, mixed with the citrate solution, was centrifuged, and the citrate solution removed from the corpuscles by means of pipettes. The corpuscles were washed twice with physiologic salt solution and the clear fluid pipetted off. The leukocytes formed a thin white layer on the surface. The leukocytes were suspended in a small amount of physiologic salt solution. The bacterial suspension was prepared from 24-hour cultures on blood agar. A number of strains were tested and two selected which seemed to represent an average susceptibility to

phagocytosis. These two strains were used throughout the series of tests made. Clumps were removed from the suspensions by centrifugalization.

The opsonic index was determined by comparing the action of normal horse serum with serum from the horses immunized with streptococcus cultures. The normal serum was obtained from a horse treated with diphtheria toxin for the production of diphtheria antitoxin. The same horse was bled in all cases and at the same time, when the immune blood was drawn. The two sera were kept together under identical conditions. As the process on concentration takes about two weeks, the concentrated sera were compared with normal serum obtained two weeks previously and kept on ice in the meantime. The antigonococcic serum was compared with the same horse serum which was used in experiment 1. A laboratory strain of the gonococcus was used and the figure obtained (0.82) is of comparative value only, inasmuch as the strain used was different from the one used by the manufacturers for preparation of the serum. In determining the opsonic index of the concentrated sera it was found that the opsonic content was so high that a single cell would take up more organisms than could be counted with accuracy. For this reason the concentrated sera were diluted with salt solution. The opsonic preparation was made by using a Pasteur pipette. A mark was made on one of these pipettes and successive portions of serum, bacterial suspension, and leukocytic suspension drawn up to this mark, separating the various portions by air bubbles. The three portions were mixed, the tip sealed, and the preparation incubated for 20 minutes at 37° C. The contents were then spread evenly over a number of glass slides, dried in the air, fixed by gentle heating, and stained with methylene blue. In making a set of preparations the same bacterial suspension and the same leukocytic suspension were used for the whole series. The opsonic index was obtained by counting the bacteria found within 200 phagocytes. Four preparations were made of each serum, so that 800 phagocytes were counted for each test.

The first experiments with concentration were made with small amounts of antistreptococcic and antigonococcic serum. They

were not heated, and the first precipitate, which is relatively small, was discarded. The second experiment was made with antistreptococcic serum only. This lot was not heated, but both precipitates were carried through. The third experiment was made with antistreptococcic serum and this was heated, and both precipitates carried through. The results appear in the following table.

No. of Experiment	Kind of Serum	Volume of Blood in Oxalate Solution	Volume of Plasma	No. of Concentrated Serum	Volume after Concentration	Opsonic Index before Concentration	Opsonic Index after Concentration	Concentration of Opsonins	Per Cent Yield of Opsonins Compared with Original Content	Loss in Filter
1...	Gonococcus	.....	960 c.c.	G 1	150 c.c.	0.82	3.000	3.655	56	40 c.c.
	Streptococcus	8,000 c.c.	3,790 c.c.	S 1	500 c.c.	2.053	7.006	3.41	45	.....
2...	Streptococcus	10,000 c.c.	5,380 c.c.	S 2	850 c.c.	2.898	13.342	4.604	67.5	275 c.c.
				S 21	460 c.c.	2.898	5.963	2.058		
3...	Streptococcus	16,000 c.c.	7,400 c.c.	S 3	670 c.c.	1.525	8.621	5.651	52	195 c.c.
				S 31	300 c.c.	1.525	5.353	3.509		

There is always some loss from manipulation in the process of concentration. This loss, however, can be reduced to a minimum by care in all details. The greatest loss occurs no doubt during filtration of the globulin solution. Considerable quantities are retained in the walls of the Berkefeld filter and a residue remains on the outside of the filter. Salt solution was passed through the Berkefeld filter after filtration of the sera. The filtered salt solution contained nearly all the serum left in the filter and this solution was incorporated with the next lot. In experiment 1 the amounts worked with were too small and we did not pass salt solution through the filter. In experiment 2 we found that the loss in the filter was 275 c.c. This amount was incorporated with the serum in experiment 3. The amount of loss was determined by deducting the filtrate from the amount obtained after dialysis. The incorporation of the remnant of experiment 2 with experiment 3 involves a slight inaccuracy in the opsonic index of serum S 3 and S 31. This inaccuracy, however, is negligible. The percentage of yield of opsonins is the amount calculated in relation to the opsonic content of the original serum. The relative loss of manipulation is larger if small amounts are worked with. In experiment 1 the

yield was 45 per cent, in experiment 2, 67.5 per cent, and in experiment 3, 52 per cent. The relatively low yield in experiment 3 is probably due to partial destruction of opsonins by heating at 56° for 15 hours.

The ratio of concentration is about the same as is obtained in concentrating diphtheria antitoxin. The antibodies in antistreptococcic and antigonococcic sera seem to be distributed among the globulins in the same relation as in diphtheria antitoxin.

The results of this work show that antistreptococcic sera and antigonococcic sera can be concentrated by the same method as diphtheria antitoxin. The globulin solution may have a potency of 3 to 5.6 times the potency of the original serum, as measured by the opsonic index. We have also shown that in these sera the chief amount of antibody is united with the globulin fraction of horse blood, the same as in diphtheria and tetanus antitoxins, and with the antibodies to Rocky Mountain spotted fever.

The clinical efficiency of the concentrated sera is being tested and the results will be published later.